ANALYSIS OF ALCOHOL IN AQUEOUS AND BIOLOGICAL SAMPLES BY HEADSPACE GAS CHROMATOGRAPHY

Introduction:

Alcohol in the vapor phase above a liquid containing alcohol will be a function of the temperature of the liquid phase, and the concentration of alcohol in the liquid phase. This procedure measures the alcohol concentration in the vapor phase or headspace above a blood or water sample in a sealed vial. An internal standard, n-propanol, is included for quantitative calculations. Sodium chloride is added to the sample to increase the partial pressure of ethanol and n-propanol. The vapor is analyzed by gas chromatography, and the relative areas of the peaks and thus the alcohol concentration, calculated by a computer. Gas chromatography separates out ethanol from any other volatile substances that might be in the sample. To avoid problems with co-elution of volatiles, each sample is analyzed on two separate systems. Results are reported in units of grams of alcohol per 100mL of whole blood, as required by the Washington Administrative Code (WAC 448.14)

Equipment:

Hewlett Packard 7694 headspace gas autosampler or equivalent
Hewlett Packard 6890 gas chromatograph or equivalent
Compressed gases; air, nitrogen, hydrogen
Computer
Autosampler vials
Pipettes
6 foot glass GC columns
30m DB-ALC1 capillary column or equivalent
30m DB-ALC2 capillary column or equivalent
Diluter

Reagents:

ethanol
n-propanol
sodium chloride
laboratory grade water
College of American Pathologists (CAP) alcohol controls
Carbopak 60/80 packing with 0.8%THEED or 0.2% Carbowax 1500 (Supelco)

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Preparation:

1) Prepare ethanol standards as described in WSTX-BKL-3.

2) Prepare internal standard solution by adding 10g sodium chloride and 0.3mL n-propanol to 2 Litre of water. Mix thoroughly and store in sealed container.

Analysis:

1) ***Handle all biological samples with care and as treat as potentially infectious***.
Inspect blood sample. Blood samples should be mobile. All clotted blood samples should be homogenized in a tissue grinder before being aliquoted for analysis.
Auto-pipette 200 µL of blood, control, or standard solution into a 10mL autosampler vial.
Add 2mL of internal standard solution.
Seal the vial and shake well until homogeneous.
Prepare each sample in duplicate.

2) Prepare vials with the following standards:
0, 0.079, 0.158, 0.316 g/100mL .

Prepare vials with a CAP ethanol control, of 0.10 or 0.20g/100mL.

3) Place the autosampler vials in the holes, on top of the spacer rings.
Use the inner numbers for location identification.

4) AUTOSAMPLER CONDITIONS:
See attached method listing. See appendix 1 for guidance in operation of Chemstation computer. Additional information may be found in the company's reference manuals.

5) GC Set-up:

System 1: 6ft glass column packed with 0.8% THEED on 60/80 Carbopak C (Supleco)

System 2: 6ft glass column packed with 0.2% Carbowax 1500 on 60/80 Carbopak C (Supelco)

System 3: 30m DB-ALC1 capillary GC column (0.53mm i.d., 3µm film)

Gas flows and oven temperatures should be set such that ethanol is resolved from all other peaks in a mixture containing methanol, ethanol, acetone, isopropanol and n-propanol (Istd). All components should be eluted in under five minutes.

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6) Calibrate the gas chromatograph using the standards; 0.079g/100mL, 0.158g/100mL, 0.316g/100mL, and a blank. Alternatively, the calibration of the gas chromatograph can be checked, and if each standard is correct to within ±0.01g/100mL of the prepared concentration, the instrument is still in calibration and may be used without recalibration.

7) Analyze CAP commercial ethanol control. Results must fall within and inclusive of ± 0.01g/100mL of the reference value. If not, recalibrate the GC and repeat the analysis. Follow the analysis of the commercial control with a blank sample containing no ethanol.

8) Repeat the analysis of the commercial control standard periodically throughout the run, followed by a blank. Each positive sample should be separated from a commercial control and blank by no more than ten other samples.

9) Analyze positive samples in duplicate, once on each of the two systems. Duplicates must agree to within ±0.010 from the mean (inclusive). Report the average of the two values, rounding to two decimal places.

10) Samples with concentrations greater than 0.400g/100mL should be diluted appropriately and re-analyzed, alternatively another suitable standard maybe run, with a result which is correct to within ±0.01g/100mL of the prepared concentration, in whhich case dilution would not be necessary.

11) Remove the metal seals from the autosampler vials. Dispose of the contents and set aside for washing. Small volumes of biological samples (less than 10mL) may be disposed of by mixing with bleach solution and emptying down the sink, followed by a rinse. Larger sample volumes will be discarded in accordance with current biological waste disposal procedures.

Documentation:

Note the type of container in which the blood sample was received. Note any unusual characteristics of blood samples. Retain chromatograms and data from all injections used to calibrate the GC, results from commercial standards, and results from the samples. Retain copies of chain of custody documentation. File with appropriate case number.

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Interpretation of results:

Post mortem samples: Blood alcohol results of 0.019g% or less shall be reported as negative. Samples drawn from living subjects: Blood alcohol results of 0.009g% or less shall be reported as negative, in accordance with WAC 448.14.

The following clinical effects and symptoms are associated with various blood alcohol levels (Caplan, 1982).

BAC (g%)	***clinical effects and symptoms***
0-0.06	no apparent influence by ordinary observations; Slight changes detectable by special tests
0.03-0.12	euphoria, sociability, decreased inhibitions, diminished attention, judgement and control, loss of efficiency in performance tests
0.09-0.25	emotional instability, loss of critical judgement, decreased sensitory response, impaired memory and comprehension, some muscular incoordination, decreased reaction time.
0.18-0.30	disorientation, mental confusion, dizziness, loss of emotional control, impaired balance, muscular incoordination, slurred speech decreased pain perception
0.27-0.40	apathy, inertia, marked decrease to stimuli and advanced muscular incoordination, vomiting, incontinence, sleep or stupor
0.35 and above	partial or complete unconsciousness, coma, respiratory distress, circulatory failure, possible death

The signs and symptoms reported at all levels may significantly impair driving regardless of their severity or detectability. Note that tolerence to alcohol such as that present in alcoholics or conditioned drinkers can cause these effects to be less obvious in some individuals.

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References:

Y.H. Caplan in "Forensic Science Handbook vol. 1." R. Saferstein (ed.) Prentice Hall , 1982.

"Goodman and Gilman's the Pharmacological Basis of Therapeutics", McMillan publishing, 7th edn., 1985

Hewlett Packard 7694 Headspace Autosampler reference manual

Hewlett Packard 6890 Gas Chromatograph reference manual

STATEMENT OF STATE TOXICOLOGIST

In my capacity as Washington State Toxicologist, and by my authority outlined in RCW 46.61.506, I have reviewed this protocol and find it to be proper and adequate in form and substance for the purpose it was intended. I therefore approve and authorize its use. This protocol replaces all previous headspace GC analysis protocols.

Barry K. Logan Ph.D., DABFT
Washington State Toxicologist

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Appendix 1.

Guidance for operation of Chemstation computer.

The principle of operation is as follows: You load a blank formatted sequence with the calibration parameters, method names, number of injections per vial already loaded. You then enter your name as the operator (this will appear on the report), and the subdirectory where the files should be sent. For convenience you should use the date formatted as (e.g. 951228 for 12/28/95). For successive runs on the same date use 951228.1 etc. You then enter your sample numbers into the sample table, and delete any additional lines if you have less than 44 samples. You then save the sequence as your name, and start the sequence. This only starts the GC which will then prepare itself for the first injection from the autosampler. It does not start the autosampler.

You then tell the autosampler how many samples there are, and start the autosampler.

Every time you want to run samples, follow this protocol. After the first time, the system will ask you if you want to overwrite the sequence <your name> when you try to save it. Say yes.

You will be unable to overwrite or alter the sequence acal1blk.s or acal2blk.s. These will always look the same (i.e. blank) when you load them.

Directions:

First load all sample vials into the autosampler.

1. Open Instrument 1 or 2 online.
   It is recommended that you complete the process for one instrument, then do the same for the second.

2. Load sequence.
   Choose acal1blk.s for instrument 1
   Choose acal2blk.s for instrument 2.

3. Open sequence parameters.
   Enter operator name
   Enter subdirectory name

4. Close sequence parameters (click on OK)
   OK to create new subdirectory? (Click on OK.)

5. Open sequence table
   enter sample names
   delete any blank lines up to 44.

6. Close sequence table
   Go to Sequence; 'save sequence as'
   Save sequence as <your name>

8. Open sequence table
   Run sequence

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7. Open Headspace Autosampler controller
   Check paper in the printer
   Click on monitor/start/chain
   Click on vial icon
   Set number of vials
   Click on download
   Click on **start**

8. Any time after the calibrators have been run, you should go back to instrument control,

   View:
   Data analysis,
   Calibration;
   Calibration table;
   Print table.

   Print Sequence
   All.

Keep calibration table, plots, and a copy of sequence table with the first sample run that day.

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